Current genetic status of common carp (*Cyprinus carpio* L.) introduced into Tunisian reservoirs

by

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ABSTRACT. - In order to enhance and develop inland fisheries and to create exploratory ichthyic stocks in reservoirs, the introduction of freshwater fishes in Tunisia began since the 1960s. However, stocking programs are usually conducted without any following assessment of the potential success of the exercise. Within the context of studying the genetic status of introduced populations, this paper describes the genetic variation of two domesticated varieties of common carp, scaly and mirror, introduced into five Tunisian reservoirs using six allozyme and four microsatellite loci. Although the genetic diversity observed with microsatellites was higher than that observed with allozyme markers, it remained lower compared to wild European populations. The lowest genetic diversity was observed in the mirror carp population of Nebhana reservoir. Both marker types highlighted significant heterogeneity among the two mirror carp populations and between the two varieties except between the two sympatric scaly and mirror carp populations of Sidi Salem reservoir. Nebhana population was found to be the most differentiated and this can be explained by genetic differences that already exist between the introduced populations, and which were increased by genetic drift and/or selection. Within scaly carp populations, homogeneity was revealed only between populations of the Mellegue and Bir M'cherga reservoirs. The significant differentiation of the scaly carp population of Sidi Salem reservoir might be due to hybridization with mirror carp sympatric in this reservoir.

RÉSUMÉ. - Statut génétique actuel des populations de la carpe commune (*Cyprinus carpio* L.) introduite dans les retenues de barrages tunisiens.

Dans le but de valoriser et de développer les pêcheries continentales en Tunisie, des introductions de poissons allochtones dans les lacs de barrages ont commencé dans les années 60. Toutefois, ces essais n'ont pas toujours été suivis d'une évaluation du potentiel de succès de l'exercice. Afin d'étudier le statut génétique des populations introduites, nous avons analysé la variabilité génétique de deux variétés de carpe commune sur six loci allozymiques et quatre loci microsatellites. Les résultats montrent que, bien que la diversité génétique révélée par les microsatellites dans les populations tunisiennes soit plus élevée que celle qui a été mise en évidence par les allozymes, elle demeure inférieure à celle qui est observée dans les populations sauvages européennes. La population de carpe miroir de Nebhana présente la plus faible diversité génétique. Les deux marqueurs révèlent une hétérogénéité significative, d'une part, entre les deux populations des carpes miroirs et, d'autre part, entre les populations des deux variétés à l'exception des carpes sympatriques de Sidi Salem. La différenciation la plus importante est celle de l'échantillon de Nebhana. Elle pourrait être expliquée par une éventuelle différence génétique initialement présente entre les populations introduites et qui s'est accentuée par dérive génétique et/ou sélection. Les valeurs de F_{ST} par paire d'échantillons montrent une homogénéité seulement entre les populations de carpe à écailles de Mellegue et Bir M'cherga. La variabilité de l'échantillon des carpes à écailles de Sidi Salem semble être la conséquence d'une hybridation avec les carpes miroir sympatriques dans le même lac de barrage.

Key words. - Cyprinidae - *Cyprinus carpio* - Genetic variation - Allozyme - Microsatellite - Stocking.

Stocking and introduction of fish are a fishery management used to improve the quantity or quality of catches and to have a long-term beneficial effect on fish stocks (Cowx, 1994). Such human activity has important economic, social and ecological benefits (Gurung, 2005). However, species introduction is considered as a balancing act where potential benefit must offset potential risk (Gurung, 2005).

In Tunisia, stocking and breeding of freshwater fishes began since the 1960s in order to enhance and develop inland fisheries and to create exploratory ichthyic stocks in reservoirs. The introduction of various non-native species, common carp (*Cyprinus carpio* Linnaeus, 1758), roach (*Rutilus rubilio* Bonaparte, 1837), rudd (*Scardinius erythrophthalmus* Linnaeus, 1758), northern pike (*Esox lucius* Linnaeus, 1758), pike-perch (*Sander lucioperca* Linnaeus, 1758), largemouth bass (*Micropterus salmoides* Lacepède, 1802), rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792), tench (*Tinca tinca* Linnaeus, 1758) and tilapia (*Oreochromis niloticus* Linnaeus, 1758), was carried out by the "Office national des Pêches" (ONP) and the "Institut national scientifique et technique d'Océanographie et de Pêche" (INSTOP) (Zaouali, 1981). In the 1990s, a Tuniso-German project (Tuniso-German coop-

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Table I. - History of common carp introductions in Tunisia.

Common carp variety	Year of introduction	Origin	Locality of introduction	Reservoir		Number of introduced individuals
				Area (ha)	Volume (10^6 m^3)	
Scaly carp	1990	Germany	Sidi Salem reservoir	4300	555	2500
			Mellegue reservoir	1050	120	1300
			Bir M'cherga reservoir	705	52.9	2500
			Sidi Saad reservoir	1710	209	50
Mirror carp	1965	Germany	Ain Salem station		–	20
	1966-1967	France				6000
	1967	Not mentioned	Nebhana reservoir	532	66.55	73
	1964	Not mentioned	Mellegue reservoir	1050	120	Not mentioned

eration of Deutsche Gesellschaft für Technische Zusammenarbeit "GTZ") was carried out with the aim to create fish stocks in reservoirs for economic purposes (Losse *et al*., 1991). Within this context, new introductions were realized and several species were introduced.

Common carp was one of the first introduced species. It has a long history of domestication and numerous strains and breeds have been developed from its ancestor, the wild common carp, in Europe as well as in Asia (Balon, 1995; Kohlmann *et al*., 2003). The first stocking of European common carp in Tunisia was undertaken in 1965-1967 when mirror carp (6,020 individuals), originating from Germany and France, were introduced by the INSTOP (Rhouma, 1975). These carps were probably the origin of the current stock present in the Sidi Salem reservoir. Another mirror carp of unknown origin was stocked in the Nebhana reservoir (73 individuals) in 1967 and in the Mellegue reservoir in 1964 by the ONP (Vincke, 1983; Losse *et al*., 1991). However, in 1982, the Mellegue reservoir was drained. This could explain the current absence of mirror carp in this reservoir (Losse *et al*., 1991). In 1990, under the GTZ project, a second variety of common carp, scaly carp originating from Germany, was introduced after an artificial selection according to size (Losse *et al*., 1991) in four different Tunisian reservoirs (Sidi Salem: 2,500, Bir M'cherga: 2,500, Mellegue: 1,300 and Sidi Saad: 50 individuals; Tab. I). The effect of such introductions on population genetic variability and differentiation, especially when low numbers of founders were introduced, has not usually been taken into consideration.

In order to study the genetic status of introduced populations in Tunisia and to identify the genetic impacts of such management, the present work was conducted by analyzing the genetic variability of six populations of the two domesticated common carp varieties collected from five Tunisian reservoirs where they were introduced about 40 years (in case of mirror carp) and 16 years ago (in case of scaly carp), using allozyme and microsatellite markers. Since no genetic data of the originally introduced individuals are available, the current genetic status of Tunisian carp could only be evaluated and discussed by comparisons with published literature data on European and Asian wild and domesticated common carp populations.

MATERIALS AND METHODS

Common carp sampling

Six populations of introduced domesticated common carp were sampled from five Tunisian reservoirs: Nebhana, Sidi Salem, Sidi Saad, Mellegue and Bir M'cherga (Fig. 1). Fishes were caught with multi meshes nets with 25-40 mm of mesh diameter from July 2006 to March 2007. The Sidi Salem reservoir hosts both varieties of domesticated common carp, mirror and scaly (Losse *et al*., 1991). They were considered as two distinct populations and sorted according to morphological criteria (presence/absence and arrangement of scales on the body and along the lateral line) set by Hollebecq and Haffray (1994). The sampling included a total of 250 individuals (176 scaly carps and 74 mirror carps) with sizes ranging between 28 and 55 cm. For allozyme analyses, muscle and liver were collected and stored at -20°C.

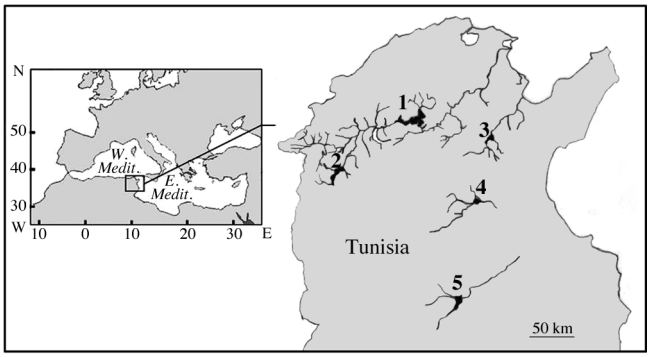

Figure 1. - Sampling localities of Tunisian common carp: (1) Sidi Salem reservoir, (2) Mellegue reservoir, (3) Bir M'cherga reservoir, (4) Nebhana reservoir, (5) Sidi Saad reservoir.

For microsatellite analyses, fins were collected and stored in 95% ethanol.

Allozyme genotyping

Two samples of 1 cm^3 from skeletal muscle and liver, respectively, were taken from each fish and homogenized with an Ultra-Turrax in an equal volume of extraction solution (Tris buffer, pH 6.8). The homogenates were centrifuged at 13,000 rpm for 30 min at 4°C. Supernatants, containing soluble proteins, were stored at -20°C until electrophoresis. Horizontal starch gel electrophoresis was carried out with the buffer system Tris-citrate, pH 8.0 described by Pasteur *et al*. (1987). Four enzymes representing nine loci were examined (Tab. II). Staining procedure was carried out according to Pasteur *et al*. (1987). Loci and alleles were named based on the nomenclature proposed by Shaklee *et al*. (1990).

Table II. - Enzymes examined, loci detected and tissues used (M = muscle; L = liver). E. C. No.: Enzyme Commission Number.

Enzyme	Abbreviation	E. C. No.	Loci	Tissue
Esterase	EST	3.1.1.1 3.1.1.2	*EST-1**	L
			*EST-2**	L
			*EST-3**	L
			*EST-4**	L
Phosphoglucomutase	PGM	2.7.5.1	*PGM-1**	M/L
Malate dehydrogenase	MDH	1.1.1.37	*MDH-1**	M/L
			*MDH-2**	M
			*MDH-2**	L
Superoxide dismutase	SOD	1.1.1.14	*SOD-1**	M/L

Microsatellite genotyping

Genomic DNA was isolated from fin clips using the peqGOLD Tissue DNA Mini Kit (Peqlab Biotechnologie). The microsatellite variation was examined at four loci (*MFW1*, *MFW6*, *MFW7* and *MFW28*) that were PCR-amplified on a Mastercycler gradient apparatus (Eppendorf) with primer sequences as described by Crooijmans *et al*. (1997). Each reaction mix was composed by 1.5 μl of 10x PCR buffer with $(NH_4)_2SO_4$ (MBI-Fermentas), 1.2 μl of 25 mM $MgCl_2$, 1.2 μl of 1.25 mM dNTPs, 0.3 μl of each primer (10 pmol. μl^{-1}), 3 μl DNA template, 0.1 μl of *Taq* DNA polymerase (5 U. μl^{-1}; MBI-Fermentas) and sterile water up to a final volume of 15 μl. The PCR program for all loci consisted of an initial denaturation at 95°C for 5 min, 5 cycles of 1 min at 95°C, 1 min at 55°C, 1 min at 72°C followed by 35 cycles of 1 min at 90°C, 1 min at 55°C, 1 min at 72°C and a final extension for 7 min at 72°C. Each locus was amplified separately. To allow multiplex fragment analyses on a CEQ 8000 (Beckman Coulter), the forward primer of each pair was labelled with one of three WellRed fluorescence dyes (Sigma-Proligo). Genotypes were recorded using the CEQ 8000 Genetic Analysis System (Fragment Analysis module) and examined with MICROCHECKER v.2.2.3 (van Oosterhout *et al*., 2004) for possible genotyping errors due to null alleles, short allele dominance (large allele dropout) and scoring errors due to stuttering.

Genetic data analyses

Allele frequencies and genetic variability parameters (mean number of alleles per locus for each and all samples [A_m], observed [H_o] and unbiased expected [H_e] heterozygosity for each locus and mean heterozygosity) were calculated by Genetix 4.05 software (Belkhir *et al*., 1996-2004) for allozyme and microsatellite genotypes. Statistical analyses (one-way ANOVA and Kruskal-Wallis test) using the SPSS10 program (SPSS, Chicago, IL) were performed to test the differences between populations in mean number of alleles and expected heterozygosities at allozyme and microsatellite loci. In all cases, p values < 0.05 were taken as indicating statistical significance.

For microsatellites, allelic richness [A_r] and richness in private alleles [A_p] following a rarefaction method, which is an estimate of allelic diversity adjusted by the lowest sample size (n = 28 individuals) (Leberg, 2002), were calculated for each population using ADZE approach (Szpiech *et al*., 2008). Statistical analyses (Kruskal-Wallis test) using the SPSS10 program were also used to test the differences between populations in [A_r] and [A_p].

The bottleneck software (http://www.ensam.inra.fr/URLB) with the three available tests: the "standardized differences test", the "sign test" (Cornuet and Luikart, 1996) and the "Wilcoxon signed-ranks test" (Piry *et al*., 1999) were used with microsatellite data for detecting recent population bottlenecks. The software tests for the departure from mutation drift equilibrium based on heterozygosity excess or deficiency. Out of the three options provided, the stepwise mutation model (SMM) and the two-phased model (TPM) were applied. As recommended by Piry *et al*. (1999), the Two Phase Mutation model (TPM) with 95% proportion of the Stepwise Mutation Model (SMM) and 5% of the Multistep mutations was used.

The variation in allozyme and microsatellite allele frequencies was analyzed using estimators (f and θ) of Wright's (1951) F-statistics (F_{IS} and F_{ST}) calculated according to Weir and Cockerham (1984). f was used to estimate departure from Hardy-Weinberg Equilibrium (HWE) for each population and for allozyme and microsatellite data. To measure the degree of genetic differentiation among the different populations, Wright's F_{ST} (θ) was estimated for the pooled data set. The F-statistics and their statistical significance of departure from zero were assessed by Genetix 4.05 software (Belkhir *et al*., 1996-2004) using 1,000 permutations. Jackknife procedure was used in order to check the contribution of each locus and each population to f and θ values. All significance

Table III. - Genetic variability of six Tunisian common carp populations at six allozyme and four microsatellite loci (*N*, sample size; A_m, mean number of alleles per locus; A_r: allelic richness; A_p: richness in private alleles; H_e: expected heterozygosity; H_o: observed heterozygosity; F_{IS}: fixation index within population; P_{HW}: significance level of Hardy-Weinberg probability test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n.s. = not significant).

			Scaly carp				Mirror carp	
Marker	Locus	Parameter	Sidi Salem	Mellegue	Sidi Saad	Bir M'cherga	Sidi Salem	Nebhana
Allozyme	*EST-2**	N	30	30	29	30	15	30
		A_m	3	3	3	2	2	2
		H_e	0.158	0.270	0.194	0.210	0.186	0.282
		H_o	0.167	0.133	0.069	0.233	0.200	0.067
		F_{IS}	-0.058	0.512	0.649	-0.115	-0.077	0.767
		P_{HW}	n.s.	n.s.	**	n.s.	n.s.	***
	*EST-4**	N	25	26	26	19	12	23
		A_m	2	2	2	2	2	1
		H_e	0.393	0.483	0.449	0.478	0.431	0.000
		H_o	0.200	0.461	0.269	0.210	0.417	0.000
		F_{IS}	0.496	0.044	0.405	0.566	0.035	-
		P_{HW}	n.s.	n.s.	n.s.	n.s.	n.s.	
	*MDH-1**	N	26	26	30	30	15	30
		A_m	2	1	2	2	2	2
		H_e	0.038	0.000	0.065	0.033	0.067	0.033
		H_o	0.038	0.000	0.067	0.033	0.067	0.033
		F_{IS}	0.000	-	-0.017	0.000	0.000	0.000
		P_{HW}	n.s.		n.s.	n.s.	n.s.	n.s.
	*MDH-2**	N	26	20	29	29	13	30
		A_m	2	2	1	3	2	2
		H_e	0.265	0.050	0.000	0.132	0.271	0.096
		H_o	0.308	0.050	0.000	0.138	0.154	0.100
		F_{IS}	-0.163	0.000	-	-0.042	0.442	-0.036
		P_{HW}	n.s.	n.s.		n.s.	n.s.	n.s.
	*PGM-1**	N	30	30	30	29	11	30
		A_m	3	3	2	3	1	2
		H_e	0.129	0.128	0.033	0.163	0	0.183
		H_o	0.067	0.067	0.033	0.034	0	0.067
		F_{IS}	0.487	0.484	0.000	0.791	-	0.640
		P_{HW}	n.s.	n.s.	n.s.	**		n.s.
	*SOD-1**	N	30	30	30	30	15	30
		A_m	1	1	1	2	1	1
		H_e	0.000	0.000	0.000	0.126	0.000	0.000
		H_o	0.000	0.000	0.000	0.133	0.000	0.000
		F_{IS}	-	-	-	-0.054	-	-
		P_{HW}				n.s.		
Microsatellite	*MFW1*	N	49	35	36	55	28	45
		A_m	10	10	7	11	9	4
		A_r	9.02	8.954	6.776	9.778	9	3,994
		A_p	0.000	0.230	0.000	1,299	0.005	0,429
		H_e	0.738	0.862	0.736	0.865	0.772	0.696
		H_o	0,755	0.886	0.611	0.873	0.750	0.644
		F_{IS}	-0.022	-0.027	0.171	-0.009	0.029	0.075
		P_{HW}	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MFW28	N	48	34	36	51	28	44

Table III. - Continued.

			Scaly carp				Mirror carp	
Marker	Locus	Parameter	Sidi Salem	Mellegue	Sidi Saad	Bir M'cherga	Sidi Salem	Nebhana
		A_m	7	4	4	4	6	7
		A_r	6.958	3.996	3.998	3.999	6	6,855
		A_p	0,973	0.000	0.000	0.000	0,002	1,985
		H_e	0.721	0.668	0.506	0.681	0.744	0.818
		H_o	0.646	0.588	0.250	0.588	0.679	0.727
		F_{IS}	0.104	0.121	0.509	0.137	0.088	0.111
		P_{HW}	n.s.	n.s.	***	n.s.	n.s.	n.s.
	MFW6	*N*	49	35	36	55	28	45
		A_m	11	8	8	9	8	7
		A_r	10.102	7.956	7.933	8.361	8	6.981
		A_p	0.819	0.002	0.002	0.51	0.181	0.000
		H_e	0.861	0.827	0.724	0.799	0.857	0.812
		H_o	0.857	0.771	0.722	0.709	0.893	0.822
		F_{IS}	0.003	0.067	0.002	0.113	-0.042	-0.012
		P_{HW}	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	MFW7	*N*	50	35	36	55	28	44
		A_m	11	11	8	9	10	5
		A_r	9.938	10.486	7.554	8.258	10	5
		A_p	0.56	1.762	0.000	0.509	0.043	1
		H_e	0.852	0.802	0.791	0.831	0.863	0.784
		H_o	0.820	0.800	0.861	0.800	0.893	0.795
		F_{IS}	0.038	0.002	-0.089	0.037	-0.035	-0.014
		P_{HW}	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

values resulting from multiple comparisons were corrected for type I errors by the Bonferroni correction method using an initial significance level of 0.05 (Rice, 1989).

To infer the genetic relationships between populations, pairwise Chord Distances (*Dc*) (Cavalli-Sforza and Edwards, 1967) were calculated for the pooled data set (allozyme and microsatellite genotypes) by Genetix 4.05 software (Belkhir *et al*., 1996-2004). Then, the NEIGHBOR and TREEVIEW modules of the PHYLIP software package (Felsenstein, 1993) were run on the distance matrix to construct a neighbor-joining (NJ) tree for the pooled data set. The robustness of tree nodes was evaluated by resampling with 1,000 bootstrap replications, and the construction of a consensus tree was carried out using the CONSENSE module of the PHYLIP software package (Felsenstein, 1993).

STRUCTURE 2.3.3 software (Pritchard *et al*., 2000) was used to determine the optimal number of genetic clusters (K) and to assign individuals to specific clusters. This program uses a Bayesian model-based clustering algorithm to group individuals into populations based on allele frequency patterns. Ten independent runs of K = 1-10 were performed with a burn-in period of 100,000 and 100,000 Monte Carlo Markov Chain (MCMC) repetitions using no prior information and assuming admixture and independent allele frequencies. We used the L(K) method of determining K, where the maximum value of the mean of LnP(D) for each K is chosen. STRUCTURE outputs for each K were compiled with the software CLUMPP (Jakobsson and Rosenberg, 2007) using the Greedy K algorithm (described in Jakobsson and Rosenberg, 2007). CLUMPP aligns multiple replicate analyses of the same data set and creates an infile for the software DISTRUCT (Rosenberg, 2004), which creates a graphical representation of the mean STRUCTURE outputs for a chosen K.

RESULTS

Genetic variability within populations

Basic variability indices (number of alleles; expected and observed heterozygosity; fixation index within populations) were computed for each of the six polymorphic out of the nine studied allozyme loci (*EST-2*, EST-4*, MDH-1*, MDH-2**, *PGM-1** and *SOD-1**), and for all the four polymorphic microsatellite loci (*MFW1*, *MFW28*, *MFW6* and *MFW7*) (Tab. III). The average number of alleles per locus (A_m) ranged from 1.444 (mirror carp of Sidi Salem and Nebhana reservoirs) to 1.889 (scaly carp from Bir M'cherga reservoir) for allozyme data and from 5.75 (mirror carp of

Table IV. - Genetic variability of six Tunisian common carp populations (n, mean sample size; A_m, mean number of alleles per locus; F_{IS}, fixation index within population; H_e, expected heterozygosity; H_o, observed heterozygosity; P_{HW}, significance level of Hardy-Weinberg probability test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n.s. = non significant).

Population	Allozyme loci						Microsatellite loci					
	n	A_m	H_e	H_o	F_{IS}	P_{HW}	n	A_m	H_e	H_o	F_{IS}	P_{HW}
Scaly carp												
Sidi Salem	30	1.778	0.109	0.086	0.210	n.s.	50	9.75	0.793	0.770	0.030	n.s.
Mellegue	30	1.667	0.103	0.079	0.239	n.s.	35	8.00	0.790	0.761	0.036	n.s.
Sidi Saad	30	1.556	0.082	0.048	0.414	***	36	6.75	0.689	0.611	0.115	***
Bir M'cherga	30	1.889	0.127	0.087	0.320	***	55	8.25	0.794	0.743	0.065	n.s.
Mirror carp												
Sidi Salem	15	1.444	0.106	0.093	0.128	n.s.	28	8.25	0.809	0.804	0.007	n.s.
Nebhana	30	1.444	0.066	0.029	0.556	***	46	5.75	0.778	0.747	0.039	n.s.

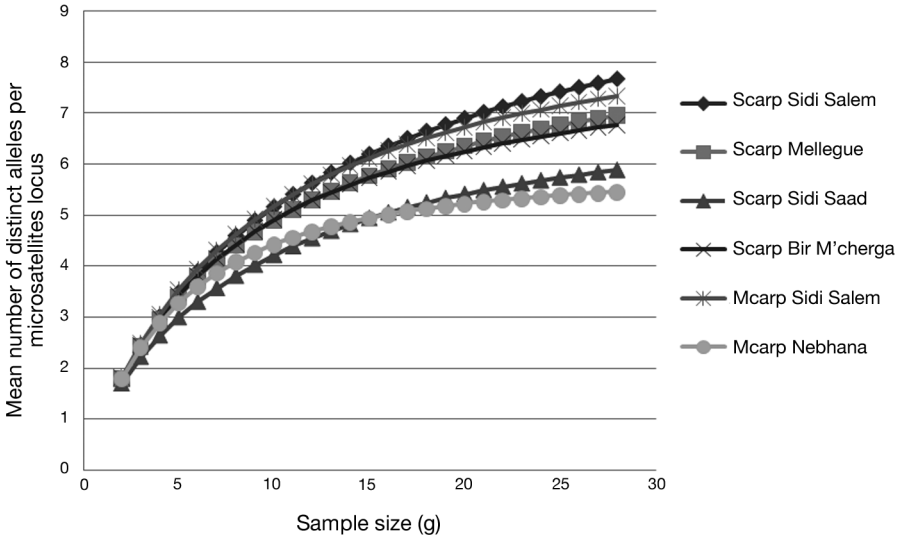

Figure 2. - Mean number of distinct alleles per microsatellite locus as a function of standardized sample size for six Tunisian common carp populations obtained by *ADZE* program. Mcarp: Mirror carp; Scarp: Scaly carp.

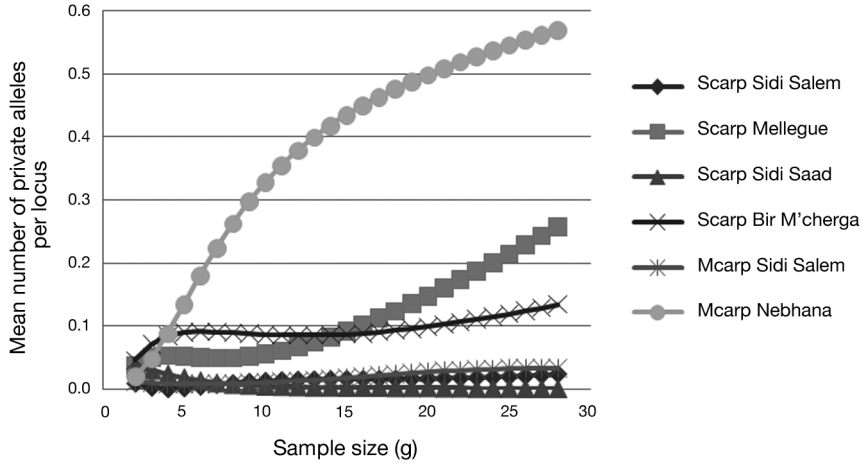

Figure 3. - Mean number of private alleles per locus as a function of standardized sample size for six Tunisian common carp populations obtained by *ADZE* program. Mcarp: Mirror carp; Scarp: Scaly carp.

Nebhana reservoir) to 9.75 (scaly carp of Sidi Salem reservoir) for microsatellite loci (Tab. IV). No statistically significant differences were found between pairs of populations (Kruskal-Wallis test, $p = 0.436$ for allozyme and $p = 0.935$ for microsatellite loci).

Allelic richness per locus, estimated after rarefaction method, for microsatellites, seem to have the same range as A_m values (Fig. 2). However, A_r and A_p showed significant differences between populations (Kruskal-Wallis test, $P = 0$ for A_r and for A_p between populations). In fact, the highest variability was observed with scaly carp in Sidi Salem reservoir and the lowest with the mirror carp population of Nebhana and the scaly carp population of Sidi Saad reservoirs (Fig. 2). The highest richness in private alleles, which is a

Table V. - Wilcoxon test for heterozygosity excess at four microsatellite loci in six Tunisian common carp populations under SMM and TPM mutation models (SMM, stepwise mutation model; TPM, two-phase model of mutation; H_e, number of loci showing heterozygosity excess; H_d, number of loci showing heterozygosity deficiency; P, statistical possibility under the Wilcoxon test that the population exhibits overall heterozygosity excess over all loci given the model: * $P < 0.05$).

Common carp variety	Population	SMM		TPM	
		H_e/H_d	P	H_e/H_d	P
Scaly carp	Mellegue	1/3	0.906	4/0	0.156
	Sidi Saad	4/0	1.000	1/3	0.937
	Bir M'cherga	1/3	0.562	3/1	0.062
	Sidi Salem	1/3	0.968	3/1	0.093
Mirror carp	Sidi Salem	2/2	0.906	2/2	0.437
	Nebhana	4/0	0.031*	4/0	0.031*

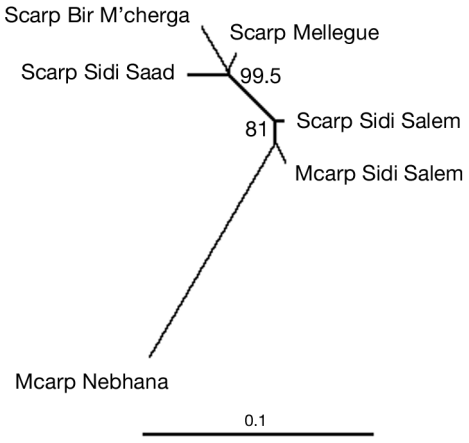

Figure 4. - Neighbour-Joining tree of six Tunisian common carp populations based on pooled data (microsatellites and allozymes) and Chord distances (Cavalli-Sforza and Edwards, 1967). Mcarp, Mirror carp, Scarp, Scaly carp.

measure of genetic distinctiveness, was observed in the mirror carp population of Nebhana reservoir (Fig. 3).

Average H_e varied from 0.066 (mirror carp of Nebhana reservoir) to 0.127 (scaly carp of Bir M'cherga reservoir) for allozyme data and from 0.689 (scaly carp of Sidi Saad reservoir) to 0.809 (mirror carp of Sidi Salem reservoir) at microsatellite loci (Tab. IV). Heterozygosity estimates for each population didn't show any statistically significant differences (ANOVA test, p = 0.939 for allozyme and p = 0.931 for microsatellite loci).

A genetic bottleneck signature was detected only in the mirror carp population of the Nebhana reservoir based on the Wilcoxon signed ranks test (p-value = 0.031; Tab. V).

Deviation from Hardy-Weinberg equilibrium

For allozymes, the scaly carp populations of Sidi Saad and Bir M'cherga reservoirs as well as the mirror carp population of Nebhana reservoir showed significant departures from Hardy-Weinberg equilibrium. The other populations appeared to be in equilibrium for all loci (Tab. III). The *EST-2** locus contributed to this departure in scaly carp of Sidi Saad ($F_{IS} = 0.649$, $p < 0.01$) and in mirror carp of Nebhana ($F_{IS} = 0.767$, $p < 0.001$) reservoirs. In contrast, the heterozygote deficit in scaly carp of Bir M'cherga reservoir was caused by the *PGM-1** locus ($F_{IS} = 0.791$, $p < 0.01$).

For microsatellites, highly significant deviations from Hardy-Weinberg equilibrium were only found at locus *MFW28* in scaly carp of Sidi Saad reservoir ($F_{IS} = 0.509$, $p < 0.001$; Tab. III). The deficiency of heterozygotes at this locus was probably caused by the presence of null allele(s) with an estimated frequency of 0.165 as indicated by the Microchecker software.

Genetic differentiation and relationships among populations

Globally, highly significant heterogeneity of the studied populations was observed for the pooled data set (allozyme and microsatellite loci) ($F_{ST\ global} = 0.068$, $p < 0.001$).

Pairwise F_{ST} values revealed significant genetic heterogeneity between most pairs of populations even after sequential Bonferroni corrections (Tab. VI). Among scaly carp, genetic

Table VI. - Matrix of F_{ST} values (above diagonal) and chord distances D_C (below diagonal) between pairs of six Tunisian common carp populations based on a pooled data set of microsatellite and allozyme loci (*Sidi Sa. MC* : Sidi Salem mirror carp, *Sidi Sa. SC* : Sidi Salem Scaly carp, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n.s. = non significant. Numbers in bold face are significant after the Bonferroni correction).

		Scaly carp				Mirror carp	
		Sidi Sa. SC	*Mellegue*	*Sidi Saad*	*Bir M'cherga*	*Sidi Sa. MC*	*Nebhana*
Scaly carp	*Sidi Sa. SC*	–	0.023**	**0.049*****	**0.042*****	0.000 n.s.	**0.089*****
	Mellegue	0.042	–	**0.033****	0.013 n.s.	0.028**	**0.121*****
	Sidi Saad	0.047	0.027	–	0,030**	**0.053*****	**0.182*****
	Bir M'cherga	0.057	0.029	0.039	–	0.036**	**0.151*****
Mirror carp	*Sidi Sa. MC*	0.030	0.058	0.064	0.064	–	**0.085*****
	Nebhana	0.115	0.149	0.170	0.180	0.118	–

homogeneity was only found between the populations of Mellegue and Bir M'cherga reservoirs (Tab. VI). In contrast, among mirror carp, the two populations of Nebhana and Sidi Salem reservoirs were significantly differentiated from each other (F_{ST} = 0.085; p < 0.001). Moreover, comparing populations of the two varieties, mirror carp of Nebhana reservoir were highly differentiated from all other studied populations (Tab. VI). However, the mirror carp of Sidi Salem reservoir were homogeneous only with the sympatric scaly carp population of the same reservoir.

The D_C genetic distances between populations based on pooled data ranged from 0.027 (between the scaly carp populations of Mellegue and Sidi Saad reservoirs) to 0.115-0.180 (between the mirror carp of Nebhana reservoir and all other populations) (Tab. VI). The NJ tree based on D_C genetic distances (Fig. 4) supports the clustering of the three scaly carp populations of Sidi Saad, Mellegue and Bir M'cherga reservoirs (99.5% bootstrap value) and the evident distinction of Nebhana population. Moreover, a close relationship of the two sympatric scaly and mirror carp populations of Sidi Salem reservoir was also shown.

The estimated log probability of the data [Ln P(D)] plateaued between K = 2 and K = 4 (Fig. 5).The structure analysis with optimal K-value (K = 2) showed that, with few exceptions, all individuals had high probabilities of belonging to their putative variety (Fig. 6). In fact, the scaly carp populations of Sidi Saad and Bir M'cherga, and the mirror carp population of Nebhana reservoirs were split into two distinct clusters with average Q ≥ 0.80. In contrast, the two populations of the two varieties of common carp, scaly and mirror, sympatric in Sidi Salem reservoir contained more admixed genotypes with individuals assigned partly to scaly and mirror carp, with average Q < 0.50. Moreover, the scaly carp population of Mellegue reservoir had some individuals assigned to the mirror carp variety. Two hypotheses can explain the presence of such individuals. The first is the fact that this reservoir was not completely drained in 1982, and some mirror carp already introduced in the 1960s could have survived. The second is that both reservoirs (Sidi Salem and Mellegue) are built on the Mejerda river, and mirror carp could have migrated upstream to Mellegue reservoir.

DISCUSSION

Genetic variability within populations

The genetic variability of Tunisian common carp was considerably higher for microsatellite markers (A_m ranged from 5.75 to 9.75; H_e ranged from 0.689 to 0.809) compared to allozymes (A_m ranged from 1.4 to 1.8; H_e ranged from 0.066 to 0.106). This difference could be expected based on known higher mutation rates reported for microsatellite loci (Dallas, 1992; Weber and Wong, 1993).

The comparability of the presented results to other population genetic studies on wild common carp for allozyme data is limited due to differences in enzymatic systems examined and electrophoretic methodology applied. In general, genetic diversity parameters of the Tunisian common carp populations are lower compared to wild carp populations ($A_{m\ Danube}$ = 1.7, $H_{e\ Danube}$ = 0.191, Murakaeva *et al.*, 2003). However, they are comparable to the genetic variability level found in domesticated European and Central Asian carp populations (A_m= 1.5, Kohlmann and Kersten, 1999; A_m = 1.4-1.7, Murakaeva *et al.*, 2003). These observa-

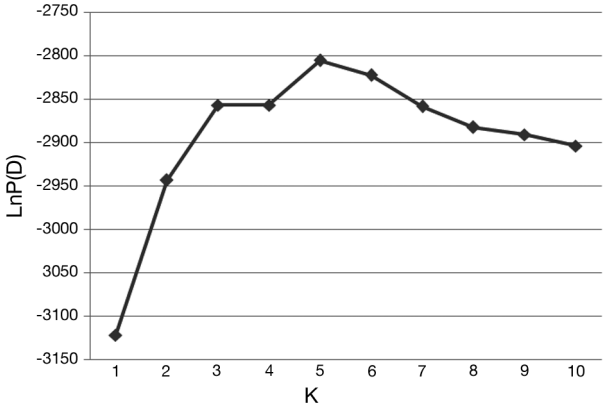

Figure 5. - Plot of the log probability of the data [LnP(D)] given values for K of 1-10.

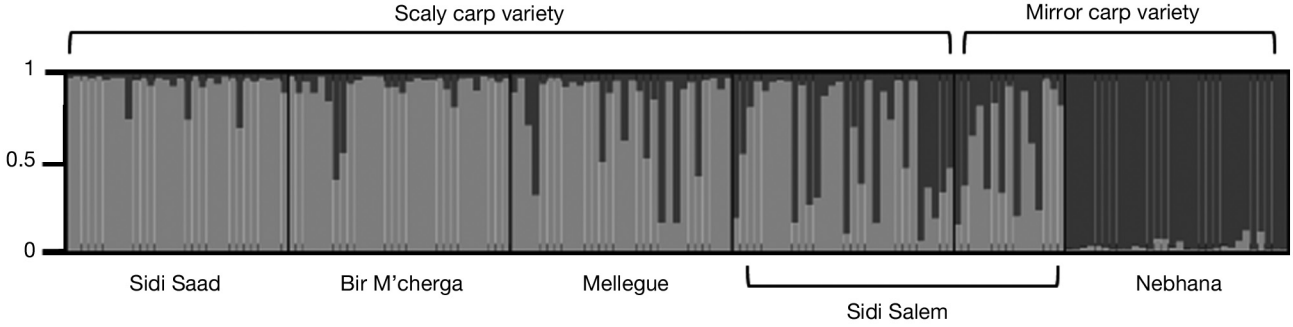

Figure 6. - STRUCTURE analysis results with *K* = 2 visualized with CLUMPP/Distruct. Each greyscale tone represents a different cluster and black lines delimit populations.

tions are in accordance with the fact that the introduced carp in Tunisian reservoirs have a domesticated origin (Vincke, 1983; Losse *et al*., 1991).

The decrease of genetic variability in domesticated carp populations compared to wild ones is better shown with microsatellite markers. In fact, using the same four microsatellite loci *MFW1*, *MFW6*, *MFW7* and *MFW28*, lower levels of genetic diversity have been observed for the present samples and for domesticated carp from Europe and Central Asia compared to wild ones (A_m varied between 5.25 and 14.25; H_e ranged from 0.610 to 0.895) (Kohlmann *et al*., 2005; Memiş and Kohlmann, 2006).

The decrease of allelic diversity in domesticated stocks compared to wild populations could have been the combined result of aquaculture practices: application of selective breeding regimes, small population sizes (Desvignes *et al*., 2001), inbreeding and/or genetic drift (Jewel *et al*., 2006).

Different authors have stressed the reduced genetic variability of captive stocks not only for common carp (Thai *et al*., 2006, 2007; Lehoczky *et al*., 2007), but also for other freshwater species such as *Salmo clarki* (Allendorf and Phelps, 1980; Leary *et al*., 1985) and *Salmo trutta* (Vuorinen, 1984). Loss of genetic variation was also observed in hatchery reared marine species such as *Crassostrea gigas* (Hedgecock and Sly, 1990), *Mercenaria mercenaria* (Dillon and Manzi, 1987), *Haliotis iris* (Smith and Conroy, 1992) and *Dicentrarchus labrax* (Bahri-Sfar *et al*., 2005).

The present study revealed statistically significant differences in genetic diversity between the mirror carp population of Nebhana reservoir and the scaly carp population of Sidi Saad reservoir and all other studied samples in allelic richness, which is not the case of the other genetic variability parameters. This statistical difference is due to the fact that expected heterozygosity is less sensitive to the presence of rare alleles and that population bottlenecks reduce allelic richness faster than heterozygosity (Kalinowski, 2004). Thus, the reduction of genetic variability seems to occur in these two populations. In fact, they have the lowest number of founding individuals.

Significant departure from Hardy-Weinberg equilibrium was observed corresponding to heterozygote deficiency at the *EST-2** and *PGM-1** allozyme loci and at the microsatellite locus *MFW28*. The possible presence of null alleles is the main hypothesis, which explains this heterozygote deficit. However, selection against heterozygous genotypes can also be advanced since allozyme loci can be under selection as reported by Ayala *et al*. (2002) and Piccino *et al*. (2004).

Genetic differentiation between populations

Strong and significant genetic differentiation was observed between mirror carp of Nebhana reservoir and all other studied populations (mirror carp of Sidi Salem reservoir and the various scaly carp). These genetic differences could already have existed between the original populations used for introduction, since mirror carp of different origin were stocked in Nebhana and Sidi Salem reservoirs in different years (separate cluster for varieties populations as showed by structure analysis). In fact, significant genetic differentiation between many European mirror carp strains has been demonstrated by Kohlmann *et al*. (2005). Then, after introduction, already existing differences could have increased due to genetic drift and/or selection. Another contributing factor to the observed significant differentiation could be the low number of founders of the mirror carp population in Nebhana reservoir (only 73 individuals were introduced by the ONP in 1967) as indicated by the positive bottleneck signal for this population and the highest value of private allelic richness. Also, the absence of the allozyme allele *EST-4*90* and the low allelic richness at microsatellites *MFW1* and *MFW7* in this population could be attributed to a combined effect of population bottleneck and genetic drift (Broders *et al*., 1999; Smith, 1995), especially if there is a total absence of migration. Finally, a selection hypothesis cannot be excluded since esterase loci can be under selection (Ayala *et al*., 2002; Balakirev *et al*., 2002)

A low degree of distinction was observed for Sidi Saad reservoir sample from all other scaly carp populations. This can be explained by the process that preceded the introduction of the few founders (only 50 individuals were introduced after a selection according to size) and given the recent introduction of this population (the beginning of the 1990s under the GTZ project).

On the other hand, the significant differentiation of the scaly carp population of Sidi Salem reservoir might be due to hybridization with mirror carp occurring in this reservoir. Hybridization between the two sympatric scaly and mirror carp varieties of Sidi Salem reservoir is further supported by the high number of introgressed individuals for both populations (Fig. 5) as well as their close relationship as illustrated on the NJ tree (Fig. 4). Indeed, gene flow is sufficient to homogenize the two gene pools of these two varieties, what would explain the observed low value of F_{ST}. Natural and experimental hybridization between varieties of common carp has been observed previously (Mondol *et al*., 2006; Gorda *et al*., 1995). Indeed, in many countries and as an outcome of genetic improvement efforts, crossbred carp are commercially used as stocking material (Hulata, 1995).

Moreover the lack of individuals with intermediate morphology in Sidi Salem reservoir is possible because the same phenomenon was observed in hybrid zone between *Solea aegyptiaca* and *Solea senegalensis* (Ouanes *et al*., 2011)

where introgressed individuals had morphotype of *aegyptiaca* or *senegalensis*.

CONCLUSION

The current genetic status of common carp in Tunisia reflects their introduction history. With the exception of Sidi Salem reservoir, scaly carp populations still cluster together in one group irrespective of the genetic markers used (allozymes or microsatellites). In contrast, the two mirror carp populations did not cluster together what could be explained mainly by their different origin. Indication for hybridization was found between the sympatric scaly and mirror carp inhabiting Sidi Salem reservoir. The present work also demonstrates sufficient genetic variation within the common carp populations studied. A tendency towards reduced variability was observed essentially for Nebhana and Sidi Saad populations. It is recommended to further monitor these populations. If this process of reduction in variability should continue and reach critical levels with potential appearance of inbreeding depressions (for example slower growth, reduced fecundity, increased malformations) higher levels of variability could be recovered by new introductions either from foreign countries or from other Tunisian carp populations.

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